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THE AËROBIC NATURE OF INSECT TISSUE.

BY R. W. GLASER,

FOREST HILLS, MASS.

It is a well-known fact that insect tissue is aërobic, *i. e.,* tubes called tracheæ ramify the entire body and supply air directly to the cells. There is no medium, like the mammalian erythrocytes, which transfers the oxygen from the lungs to the tissues.

During the course of some work on the cultivation of insect blood cells *in vitro,* I made some observations that clearly showed insect tissue to be dependent upon direct air. Since the method could be very nicely used in entomological class demonstration work, I thought it well to present the observations. The method for preparing tissue-culture slides is familiar to all biologists, so I will not dwell on very many of the technical details.[1] Suffice it to say, that I used depression slides with thin *No. 0* cover slips so that I could work effectively with the oil immersion lens. Both army worm (*Cirphis unipuncta*) and gipsy moth caterpillar (*Porthetria dispar*) blood was found suitable for the experiments.

All work must be done under sterile conditions. The animals to be operated upon are held in one hand and bent back so that the ventral side can be washed off with alcohol. When this evaporates, the tip of a proleg is then quickly cut off with sterile scissors and the drop

[1] Those interested in the cultivation of insect tissue are referred to: R. W. Glaser, "The Growth of Insect Blood Cells *in vitro.*" Psyche, Vol. XXIV, No. 1, 1917.

of blood which oozes forth is caught on a sterile cover slip. The cover slip is then placed over a sterile depression slide, and the edges are sealed with melted vaseline.

Insect blood cells grow very well in their own plasma, but Locke's solution can be added if desired. Locke's solution is isotonic with insect tissue, but has no particular advantage except that its addition thins out the plasma and produces a greater transparency in the preparations.

If we carefully place the drop of blood in the center of the cover slip the cells grow very slowly. Many disintegrate entirely, and others do not show any visible growth for two or three weeks. Even then no large syncytial masses are formed, and sooner or later, as soon as the supply of oxygen in the depression slide is diminished, begin to disorganize. At first I supposed that the death of the cells was due to the exhaustion of the required nutrition, but such was not the case. Insect blood cells can be kept alive for a surprisingly long time without the addition of fresh media. If the preparation, in which the cells are disintegrating, is shaken a bit so that the drop of blood flows over along the edges of the cover slip a change will soon become apparent. On reëxamination after about two or three weeks the cells lying in close proximity to the vaseline will appear healthy, and beautiful large syncytial masses will be found.

In order to be certain that the vaseline was not giving off something which stimulated the cells to grow, the following experiments were performed: Blood was placed in the center of the cover slips and a small piece of sterile vaseline added to each drop. Six such slides were prepared, but I failed to notice any large syncytial masses even after six weeks. The preparations were then shaken so that the blood flowed over along the edges of the cover slips and in two to three weeks more the syncytial masses were obtained.

We can conclude from these observations that direct air is absolutely necessary for the growth of insect tissue. The blood cells kept in the center of the cover glass soon exhaust the oxygen present and cease to grow. However, if they are brought in contact with the vaseline through which the air undoubtedly filters, they become rejuvenated and form syncytia. Of course air filters through the vaseline also when the cells are confined to the center of the cover slip, but it is not so readily nor so rapidly obtained by the cells. The latter are

highly metabolic and require oxygen very rapidly. My contentions were further proven by preparing slides and sealing the edges of the cover slips with a thick ring of paraffin. The blood was made to flow over along the edges as usual, but no syncytial tissue-like masses were formed. Apparently the air was unable to filter through this thick layer of paraffin.

A drop of blood permitted to remain in the center of the cover slip usually will assume a light yellow or light brown color. However, if the preparation is manipulated in such a way as to permit the drop of blood to come in contact with the edges of the cover-slip it rapidly turns black in color. This is due to the fact that the air filters through the vaseline and the tyrosinase present in insect blood oxidizes the colorless tyrosine producing a dark pigment.

A LARGE NUMBER OF SPECIES OF BUTTERFLIES OBSERVED IN ONE DAY'S COLLECTING.

BY FRANK E. WATSON,

NEW YORK, N. Y.

This paper might be entitled Butterflies of Fort Montgomery, as it deals exclusively with those observed at that locality, except that no attempt has been made to list all the species known to occur there. The writer simply wishes to bring out what he believes to be an extraordinary number of forms observed on the wing in one day in a single locality.

Fort Montgomery, Orange Co., N. Y., is a small historic town situated on the west bank of the Hudson River, some five miles south of West Point and about forty-three miles, on an air line, north of New York City. It is practically in the heart of the Highlands, with Anthony's Nose looming up on the opposite shore and Bear Mountain a mile or two to the west.[1] The region is well watered and rather rugged; the hills are steep and attain an average altitude of about 1,200 feet; the vegetation is rich and diversified. The collecting

[1] Those interested should consult the U. S. Topographical Maps—West Point and Schunemunk Quadrangles.